

CLAIMS

1. A method of producing double low restorer lines of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting radish introgression carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour, said method including the step of:
 - a) crossing double low cms lines of spring Brassica napus comprising a deleted radish insertion with the double low line of spring Drakkar for forming heterozygous restored plants of Brassica napus,
 - b) irradiating before meiosis the heterozygous restored plants obtained in step a) with gamma ray irradiation,
 - c) crossing pollen from flowers obtained in step b) with the cms double low spring Wesroona line,
 - d) testing the progeny for vigour, female fertility and transmission rate of the cms gene,
 - e) selecting progeny lines.
2. A method according to claim 1, wherein the irradiation dose in step b) is 65 Gray during 6 mn.
3. A method according to claim 1 wherein the double low cms line of spring Brassica napus of step a) is R211.
4. A method according to claim 1 wherein the testing in step d) is performed with the combination of five markers selected from PGIol, PGIUNT, PGInt, BolJon and CP418.
5. Double low restorer lines of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a

good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

- 5 6. Double low restorer lines of Brassica napus according to claim 5, wherein they present a unique combination of five markers selected from PGIol, PGIUNT, PGIint, BoIJon and CP418.
7. Brassica napus hybrid plants and progeny thereof obtained through the steps of:
 - 10 a) providing a restorer line produced according to claim 1 and bred to be homozygous,
 - b) using said restorer line in a hybrid production field as the pollinator,
 - c) using cms sterile plants in a hybrid production field as the hybrid seed producing plant, and
 - 15 d) harvesting the hybrid seed from the male sterile plant.
8. The seeds of Brassica plant developed from the Brassica line obtained in claim 1.
- 20 9. The seeds of Brassica napus obtained in claim 7.
10. The seeds of Brassica napus obtained in claims 1 and 2 deposited in NCIMB Limited, 23 St Machar Drive, Aberdeen, Scotland, AB24 3RY, UK, on July 4 , 2003, under the reference number NCIMB41183.
- 25 11. Use of the combination of at least four markers PGIol, PGIint, BoIJon and CP418, or any portion of them comprising at least one polymorphic site, for characterising recombined restorer lines of Brassica napus for Ogura cms presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.
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12. Use according to claim 11 wherein the combination is of five markers PGIol, PGIUNT, PGIint, BolJon and CP418.
- 5 13. Use according to claim 12, wherein:
- The marker PGIol is amplified using the primers: PGIol U and PGIol L
(PGIol U: 5'TCATTGATTGTTGCGCCTG3';
PGIol L: 5'TGTACATCAGACCCGGTAGAAAA3')
 - The marker PGIint is amplified using the primers: PGIint U and PGIint L
10 (PGIint U: 5'CAGCACTAATCTTGCGGTATG3';
PGIint L: 5'CAATAACCCTAAAAGCACCTG3')
 - The marker PGIUNT is amplified using the primers: PGIol U and PGIint L:
(PGIol U: 5'TCATTGATTGTTGCGCCTG3';
PGIint L: 5'CAATAACCCTAAAAGCACCTG3')
 - 15 - The marker BolJon is amplified using the primers: BolJon U and BolJon L:
(BolJon U: 5'GATCCGATTCTTCTCCTGTTG3';
BolJon L: 5'GCCTACTCCTCAAATCACTCT3')
 - The marker CP418 is amplified using the primers: SG129 U and pCP418 L:
(SG129 U: cf Giancola et al (5)
20 pCP418 L: 5'AATTTCTCCATCACAAGGACC3')

14. PGIol marker whose sequence follows:

25 TCATTGATT GTTGCCTG TCGCCTTGTT GTGTTATGAT GAATGAACAG CAGTCATTTA 60
ACATGTGGTT AACTTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120
AATCTTGGG TATGAATTG TGATTAAATT TGTTTGTGTTG TGACTCTTC TTCATTGTTT 180
GTTTTCGTAC AATAAACCGA ATGTATAATC TTTTACAAA CTGAATTTTC TACCGGGTCT 240
GATGTACA 248

15. PGIUNT marker whose sequence follows:

30 TCATTGATT GTTGCCTG TCGCCTTGTT GTGTTATGAT GAATGAACAG CAGTCATTTA 60
ACATGTGGTT AACTTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120
AATCTTGGG TATGAATTG TGATTAAATT TGTTTGTGTTG TGACTCTTC TTCATTGTTT 180
GTTTTCGTAC AATAAACCGA ATGTATAATC TTTTACAAA TGAATTTTCT ACCGGGTCTG 240
ATGTACAATG CTAGTCTCCA TGTTCTTGGG GATCATGATT TATTTTCTAC ATGTATTTCAG 300
35 ACAGTACAGA AGAAAGTGTT CAAACTCTG GATGTTTAA TTTACAGTTA GTGGAGAAGT 360
TCGGCATTGA TCCGAACAAT GCATTTCAT TTTGGGACTG GGTGGTGGA AGGTACAGTG 420
GTAAGTGCTT GTTTATTGG TTGTATAAAT TTCTCGTCCA TTTCCGCTTG CTTAGTGTAT 480
AACTGAAATT CTTTTCAGT TTGCAGTGCT GTTGGAGTCT TACCATTGTC TCTACAGTAT 540
GGCTTCTCTG TGGTTGAGAA GTACGGTACC TTCTACTTTA TCAGCCATCT CATAAATGT 600

CTTAGGCATA TTCTTCTAT TTTATTTCCC TCTTAATGAT TTCTTCTTTT TTTTATTGCA 660
 TTCCCGTTTT ATTTTCAAAA GTTGTTACTG TCTCTAAATC AAGAAGAAAC CTTCTTAGTA 720
 GATCCAGCTG ATATTCAGCC TTTTAAAT TGGACTGCAG GTTTTAAAG GGGAGCTTCA 780
 AGCATTGATA AGCATTTCGA GTCCACACCG TTTGAGAAGA ATATACCCGT GAGTTGCATT 840
 5 AGTTGTGTGA TTATACAGTT TTCTTGTCTT TTTGCTATGT CCATCAACAC TAGAGATTCTG 900
 TGAAGTTATT AGTGTAGTCA ACGCATAGGG AGAGGTGATT GGTGACTTTT GGACGATTTT 960
 AGGTGCTTTA GGGTTATTG 979

16. PGIint marker whose sequence follows:

10 CAGCACTAAT CTTGCGGTAT GAATTTGTGA TTAAATTTGT TTGTTTGTGA CTCTTTCTTC 60
 ATTGTTCTGT TTCGTACAAT AAACCGAATG TATAATCTTT TACAACTGA ATTTTCTACC 120
 GGGTCTGATG TACAATGCTA GTCTCCATGT TCTTGGGGAT CATGATTTAT TTTCTACATG 180
 TATTCAGACA GTACAGAAGA AAGTGTTCAA AACTCTGGAT GTTTTAATTT ACAGTTAGTG 240
 GAGAAGTTCG GCATTGATCC GAACAATGCA TTTGCATTTT GGGACTGGGT TGGTGAAGG 300
 15 TACAGTGGTA AGTGCTTGTT TATTTGGTTG TATAAATTC TCGTCCATT TCGTCTGCTT 360
 AGTGATAAC TGAAATCTT TTGCAGTTTG CAGTGTCTGT GGAGTCTTAC CATTGTCTCT 420
 ACAGTATGGC TTCTCTGTGG TTTGAGAAGTA CGGTACCTTC TACTTTATCA GCCATCTCAT 480
 AAAATGTCTT AGGCATATTC TTTCTATTTT ATTTCCCTCT TAATGATTTT TTCTTTTTTT 540
 TATTGCATTC CCGTTTTATT TTCAAAGTT GTTACTGTCT CTAAATCAAG AAGAAACCTT 600
 20 CTTAGTAGAT CCAGCTGATA TTCAGCCTTT TTTAAATTGG ACTGCAGGT TTTAAAGGGG 660
 AGCTTCAAGC ATTGATAAGC ATTTCCAGTC CACACCGTT GAGAAGAATA TACCCGTGAG 720
 TTGCATTAGT TGTGTGATTA TACAGTTTC TTGTCTTTT GCTATGTCCA TCAACACTAG 780
 AGATTCGTGA AGTTATTAGT GTAGTCAACG CATAGGGAGA GGTGATTGGT GACTTTTGA 840
 CGATTCAGG TGCTTTAGGG TTATTG 866
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17. BolJon marker whose sequence follows:

GATCCGATTC TTCTCCTGTT GAGATCAGCT CCAAACATCA AACAACTTGT ACACAAATAT 60
 CTTTACTTGC TAAATGGAAC ATGACAAGAG ATAGAAAATC TTGCTCATAG TATTGTACAA 120
 GGGATAACAG TGTAGAAAAC AAACCGTCTG TAAGATTTTC TCCCTGATCC TCTCACTTAA 180
 30 CCAGTAGGCG TTTTTCACAT TGAAGCGCAT ATCTACTTTG GTATTCACTG AATAAAAAAA 240
 GAAAGCTGGT AACATGTGAA GGATATACAA GCATTGATAC ACCAAGTAGT CACAACTAC 300
 ATTATAAAGG TCAGACCTTT GTTCACATTC TGGCCTCCAG GACCACCGCT TCTAGCAAAG 360
 TTAAGCGTAA CATGGTCTGC ACGTATACAA ATGAAAATGT TTCTATCAA ATCCTATAAA 420
 ATAGAGCTCT ATAACATTGT CGATACATAG TTTCACTAAC TCTGCAAGTA CTAAACACAT 480
 35 ATACAAACAA AACTATGCGA ACAGATCAA ACTACTACAG AACACAGTTC TATGACACTG 540
 TCGATAGTAA CATCCTCTGC AAGTACCAA GAGATAGCAA ATGAAACTAT GTAAACAAAT 600
 CAAAATTCTA AATTCTCCA TCACAAGGAG CTACAGAATA GAGTTATCAT AACATTTTCT 660
 GTAAATATTT CCATCAAAAT GACTAGAGAA CAGAGTTCTT ATAACATTAT CTGTAAATGT 720
 TCCAACAAAA CCACTACATA GCAGAGTTCT TATAACATTG TCTGTAAATG TCCAATCAA 780
 40 ACCACTACAG AACAAAGCTC CTATAACATT GTTTATACAA AGTTTCACTA AATCTACAA 840
 CTTTCCCCGT AAATGAGCTT AATATCACC AAAGATGTTT CAATCAGATA AAGAGTACGA 900
 CATCGTTTTG AGATTAGAAC AAAGTGAAC TTACGTAGAG TGATTGAGG AGTAGGC 957

18. CP418 marker whose sequence follows:

45 AATTTCTCCA TCACAAGGAC CTACAGAATA GAGTTATCAT AACATTTTCT GTAAATATTT 60
 CCATCAAAAT GACTAGAGAA CAGAGTTCTT ATAACATTAT CTGTAAATGT TCCAACAAAA 120
 CCACTACATA GCAGAGTTCT TATAACATTG TCTGTAAATG TCCAATCAA ACCACTACAG 180
 AACAAAGCTC CTATAACATT GTTTATACAA AGTTTCACTA AATCTACAA CTTTCCCCGT 240
 AAATGAGCTT AATATCACC AAAGATGTTT CAATCAGATA AAGAGTAACG ACATCGTTTT 300
 50 GAGATTAGAA CAACTGAAA CTTACGTAGA GTGATTTGAG GAGTAGGCTC GTTGCCAGCA 360
 GAGCTAGCTC TCTCCTCCGC CTCATGAAGC ATCTGTTGCA CCTGAGACAA CCGTGACGAA 420
 ACTTTCCGAT CACCGCCACC AGAATTCGAC GCCGCGCATC GGAAGGATCC GAATCGGGAA 480
 CTGAGTGAAC CCGAGCGATC CCGGGAGTGC GACGGAGCGA TGGGAAAAGA GAGTGGCAG 540
 ATTTCCGCGA AGAGTGGGAG AGGAGAGGGT GGTGGATAAA CTCGCGTATG ATCAAGTTCTG 600
 55 TCATCGTCCT GATTGCCGCC ATTTTTTTT TCAGGGCGCT CTGTGGCTTA GAAGTTTCCG 660
 atgtcaatga ac 672